

## **A NOVEL DUAL-TARGETING DRUG FOR SOLID TUMORS AND THEIR VASCULATURE**

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**INTRODUCTION** Tumor growth depends on a steady blood supply. It is now widely recognized that any solid tumor with a volume of  $>1-2 \text{ mm}^3$  requires the formation of new blood vessels. It is known that tumor blood vessels express certain markers that are either present at very low levels or are entirely absent in normal blood vessels. Such markers can be used as the targets to selectively deliver therapeutic agents to tumor blood vessels and adjacent tumor cells. This dual targeting approach represents an attractive new anti-cancer strategy. NGR (asparagine-glycine-arginine) motif has been shown to bind to a specific isoform of CD13 (aminopeptidase N) which is selectively expressed on endothelial cells of tumor blood vessels (1,2). In the current report, we describe a novel dual targeting drug, TVT-Dox which is the NGR-targeted liposomal doxorubicin.

**METHODS** Evaluation of TVT-Dox binding on SLK cells by fluorescence microscopy: SLK cells grown in chambered slides were washed with PBS and incubated at  $4^\circ\text{C}$  either with TVT-Dox or Doxil diluted in PBS. After one hour, the slides were washed with PBS, mounted and evaluated for doxorubicin fluorescence under Nikon fluorescent microscope. Tumor animal models: Colorectal HT-29 and renal BB-64 and Caki-1 human tumors were implanted subcutaneously in nude Balb/C female mice. Treatment started when tumors had reached at least  $100 \text{ mm}^3$  in mean volume. Intravenous dosing regimen was 6 mg Dox/kg Q7d x 4; 5 mg Dox/kg Q7d x 5; and 6 and 9 mg Dox /kg Q7d x 4, in HT-29, BB-64, and Caki-1, respectively. Orthotopic non small cell lung carcinoma model was established by injection of H-460 cells in the pleural area of lungs. Treatment started 7 days post-inoculation with dosing regimens of 3 and 5 mg Dox /kg Q7d x 3. Therapeutic efficacy was followed by rate of tumor growth and animal survival over controls.

**RESULTS** Incubation with anti-CD13 monoclonal antibody (clone B-F10) suggests that CD13 is expressed on the membrane of SLK cells (endothelial cell origin). Preliminary immunohistochemical data indicate that TVT-Dox binds to CD13 on the membrane of SLK cells. This binding was inhibited in the presence

of the free NGR peptide, suggesting that the CD13 on SLK cells is likely to be the isoform to which the NGR motif binds specifically. In contrast, Doxil, a non-targeted liposomal doxorubicin, had no detectable binding to the membrane of SLK cells. Preliminary pharmacokinetic studies in rats indicate that TVT-Dox is very stable *in vivo* with a half-life > 24 h. In animal efficacy models, the tumor volume ratio of treated animals to controls (T/C) was used to characterize the antitumor efficacy of TVT-Dox in subcutaneous solid tumor models (colorectal and renal carcinomas). T/C values were 21%, 25%, and 23% in colorectal HT-29, renal BB-64, and renal Caki-1 models, respectively. In the case of the orthotopic H-460 lung tumor model, the increase in life span (ILS) was used as a parameter to evaluate the anti-tumor activity of TVT-Dox. The ILS value was 58 % for the highest dose tested (5 mg Dox /kg Q7d x 3).

**DISCUSSION** There are two major issues with conventional chemotherapies: 1) Poor selectivity (low drug concentration at tumor sites and distribution in normal tissues); 2) Unfavorable pharmacokinetics (rapid disappearance in the systemic circulation). Doxil, a non-targeted PEGylated liposomal doxorubicin with a long half-life time, has demonstrated certain clinical benefits over doxorubicin. However, lack of targeting mechanism has limited the use of Doxil in the clinical setting. TVT-Dox is a PEGylated liposomal Doxorubicin coated with a 19 amino acid-tumor vascular targeting peptide containing NGR motif. TVT-Dox has been shown to be capable of selective binding to endothelial cells in tumor blood vessels and effectively delivering the cytotoxic agent doxorubicin to the tumor blood vessels and adjacent tumor cells. Upon its binding to the specific CD13 isoform, TVT-Dox is internalized into tumor vascular endothelial cells and releases doxorubicin which acts as a cytotoxic agent functionally disrupting tumor vasculature. Because of the expression of the NGR-specific isoform of CD13 on some tumor cells and leakiness of tumor vasculature, TVT-Dox also selectively delivers doxorubicin to the tumor cells adjacent to the tumor vasculature. This dual-targeting mechanism differentiates TVT-Dox from traditional tumor vasculature targeting agents. With its long half-life time and dual targeting mechanism, TVT-Dox is believed to be a novel anti-cancer drug for a wide variety of solid tumors including those resistant to conventional chemotherapies.

#### **REFERENCES**

1. Pasqualini, R. et al. (2000) Cancer Res. 60, 722-727.
2. Curnis, F. et al. (2002) Cancer Res. 62, 867-874.